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Novel Insertion Sequence- and Transposon-Mediated Genetic Rearrangements in Genomic Island SGI1 of *Salmonella enterica* Serovar Kentucky[▽]

Benoît Doublet,[1,2]* Karine Praud,[2] Sophie Bertrand,[3] Jean-Marc Collard,[3] François-Xavier Weill,[1] and Axel Cloeckaert[2]

Institut Pasteur, Centre National de Référence des Salmonella, Laboratoire des Bactéries Pathogènes Entériques, 28 Rue du Docteur Roux, 75724 Paris Cedex 15, France[1]*; INRA, UR1282, Infectiologie Animale et Santé Publique, IASP, Nouzilly F-37380, France*[2]*; and National Reference Centre for Salmonella and Shigella, Bacteriology Division, Scientific Institute of Public Health, 14 Wytsman Street, B-1050 Brussels, Belgium*[3]

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***Salmonella* genomic island 1 (SGI1) is an integrative mobilizable element that harbors a multidrug resistance (MDR) gene cluster. Since its identification in epidemic *Salmonella enterica* serovar Typhimurium DT104 strains, variant SGI1 MDR gene clusters conferring different MDR phenotypes have been identified in several *S. enterica* serovars and classified as SGI1-A to -O. A study was undertaken to characterize SGI1 from serovar Kentucky strains isolated from travelers returning from Africa. Several strains tested were found to contain the partially characterized variant SGI1-K, recently described in a serovar Kentucky strain isolated in Australia. This variant contained only one cassette array, *aac(3)-Id–aadA7*, and an adjacent mercury resistance module. Here, the uncharacterized part of SGI1-K was sequenced. Downstream of the *mer* module similar to that found in Tn*21*, a mosaic genetic structure was found, comprising (i) part of Tn*1721* containing the tetracycline resistance genes *tetR* and *tet*(A); (ii) part of Tn*5393* containing the streptomycin resistance genes *strAB*, IS*1133*, and a truncated *tnpR* gene; and (iii) a Tn*3*-like region containing the *tnpR* gene and the β-lactamase bla_{TEM-1} gene flanked by two IS*26* elements in opposite orientations. The rightmost IS*26* element was shown to be inserted into the S044 open reading frame of the SGI1 backbone. This variant MDR region was named SGI1-K1 according to the previously described variant SGI1-K. Other SGI1-K MDR regions due to different IS*26* locations, inversion, and partial deletions were characterized and named SGI1-K2 to -K5. Two new SGI1 variants named SGI1-P1 and -P2 contained only the Tn*3*-like region comprising the β-lactamase bla_{TEM-1} gene flanked by the two IS*26* elements inserted into the SGI1 backbone. Three other new variants harbored only one IS*26* element inserted in place of the MDR region of SGI1 and were named SGI1-Q1 to -Q3. Thus, in serovar Kentucky, the SGI1 MDR region undergoes recombinational and insertional events of transposon and insertion sequences, resulting in a higher diversity of MDR gene clusters than previously reported and consequently a higher diversity of MDR phenotypes.**

The 43-kb *Salmonella* genomic island 1 (SGI1) is a *Salmonella enterica*-derived integrative mobilizable element that was originally identified in epidemic multidrug-resistant *S. enterica* serovar Typhimurium phage type DT104 strains (4). SGI1 contains a multidrug resistance (MDR) region containing all antibiotic resistance genes, which is a complex class 1 integron, recently named In104 and located in the 3′ part of the island (Fig. 1) (4, 25). The In104 complex integron of SGI1 confers the common multidrug resistance profile to ampicillin, chloramphenicol-florfenicol, streptomycin-spectinomycin, sulfonamides, and tetracycline of DT104 isolates (4, 10, 34). The In104 complex integron possesses two cassette attachment sites (*attI1*). At the first *attI1* site of this complex integron, the cassette carries the *aadA2* gene, which confers resistance to streptomycin and spectinomycin, and downstream, a 3′ conserved segment (3′-CS) with a truncated *sul1* gene (*sul1*Δ) is found. At the second *attI1* site, the cassette contains the β-lactamase gene bla_{PSE-1}, conferring resistance to ampicillin, and downstream, the 3′-CS comprises a complete *sul1* gene conferring resistance to sulfonamides. Flanked by the two cassettes are the *floR* gene, which confers cross-resistance to chloramphenicol and florfenicol, and the tetracycline resistance genes *tetR* and *tet*(G) (4). The In104 complex integron belongs to the In4 group of integrons, which includes an IS*6100* element downstream of the 3′-CS and is bound by the 25-bp inverted repeats IRi and IRt (36, 37).

Since the identification of SGI1 in *S. enterica* serovar Typhimurium DT104, variant SGI1 MDR regions have been described for a wide variety of *S. enterica* serovars such as serovars Agona, Albany, Cerro, Derby, Dusseldorf, Emek, Infantis, Kentucky, Kiambu, Kingston, Meleagridis, Newport, and Paratyphi B (4, 23). Recently, SGI1 and variants of it have been identified in *Proteus mirabilis* clinical and food isolates (1, 5, 13). The identification of SGI1 in *P. mirabilis* clinical isolates is of great concern, as the spread of the SGI1 MDR phenotype could have significant clinical implications in pathogenic bacteria other than *Salmonella*. SGI1

* Corresponding author. Mailing address: Centre National de Référence des Salmonella, Laboratoire des Bactéries Pathogènes Entériques, Institut Pasteur, 28 Rue du Docteur Roux, 75724 Paris Cedex 15, France. Phone: 33-(0)1 45 68 83 44. Fax: 33-(0)1 45 68 88 37. E-mail: Benoit.Doublet@tours.inra.fr.

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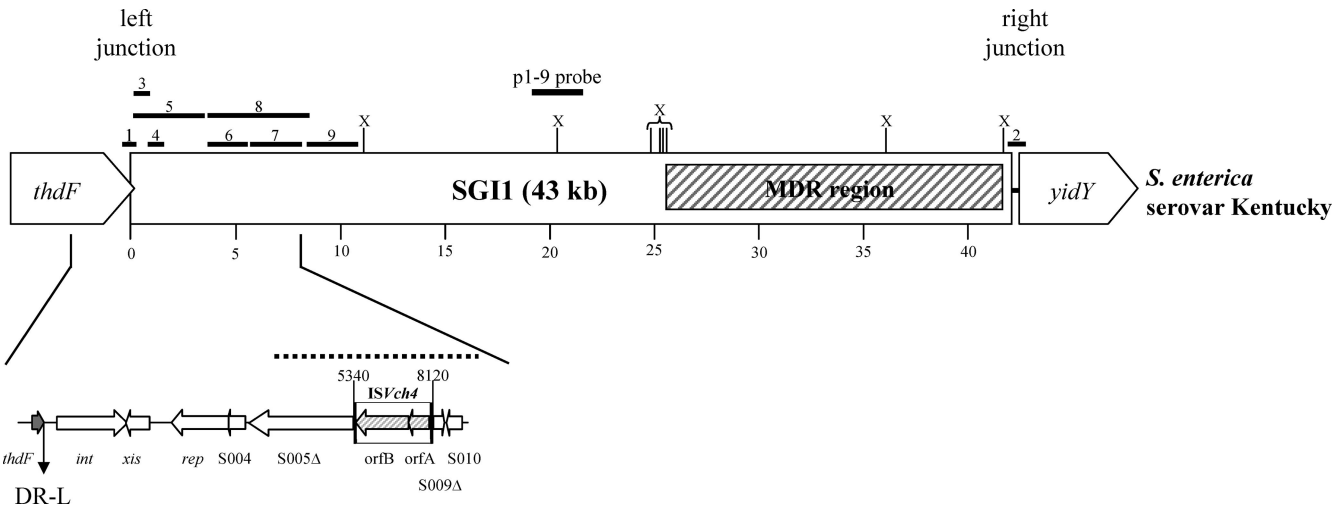

FIG. 1. Schematic view of SGI1 integrated into the *S. enterica* serovar Kentucky chromosome. The left and right junctions and the MDR region are indicated. The genetic rearrangement due to IS*Vch4* (not to scale) is shown at the bottom. DR-L indicates the left 18-bp direct repeat of SGI1. Base pair coordinates are from the complete SGI1 sequence (GenBank accession no. AF261825). PCRs carried out to map the 5′ part are indicated by thick black bars and are numbered (Table 1). The black dotted line indicates a sequenced region. X, XbaI.

variant MDR regions were accordingly classified as SGI1-A to SGI1-O (5, 9, 34, 43).

In all cases, i.e., *S. enterica* serovars and *P. mirabilis* strains, SGI1 or variants of it are found integrated into the bacterial chromosome within the last 18 bp of the *thdF* gene (4, 5, 14–16, 34). In 2005, we reported that SGI1 could be conjugally transferred from *S. enterica* donor strains to non-SGI1 *S. enterica* and *Escherichia coli* recipient strains, where it integrated into the recipient chromosome in a site-specific manner (12). Briefly, after the excision of SGI1 from the *Salmonella* chromosome, the conjugative mobilization in *trans* by a helper IncC plasmid, R55, occurs between donor and recipient strains. In the recipient cell, the circular form of SGI1 integrates in a site-specific manner at the 3′ end of the chromosomal *thdF* gene. SGI1 was thus classified within the group of site-specific integrative mobilizable elements that are related to integrative conjugative elements (12, 34).

The generation of SGI1 variant MDR regions mostly appears to be a result of events that could have occurred by homologous recombination or resistance gene cassette exchange (3, 9, 14–16). Recently, Levings et al. reported the SGI1-K variant in a multidrug-resistant *S. enterica* serovar Kentucky (hereafter referred to as serovar Kentucky) strain isolated from spice imported from India into Australia (26, 27). This variant was found to contain a complex integron with only one cassette array, *aacCA5* [also called *aac(3)-Id*, which is used hereafter] and *aadA7*, and an adjacent mercury resistance module. Moreover, part of the 3′-CS segment together with the internal additional copy of IRt has been removed, probably by an IS*6100*-mediated deletion (27). The mercury resistance region in this SGI1-K variant was found adjacent to the right-hand IRt defining the end of In4-type integrons. However, the 3′ end region of SGI1-K, estimated to be at least 10 kb in size, was not characterized (27).

Since 2002, an increasing number of multidrug- and/or ciprofloxacin-resistant strains of serovar Kentucky isolated from European travelers returning from Africa has been reported (11, 44). In the present work, we conducted a retrospective study of serovar Kentucky strains that were previously studied for resistance to quinolones (11, 44). The identification of SGI1 and characterization of new SGI1 variants were thus undertaken for these multidrug- and/or quinolone-resistant strains of *Salmonella* serovar Kentucky isolated in Belgium and France.

MATERIALS AND METHODS

Bacteria, media, and antibiotic susceptibility testing. Sixteen serovar Kentucky strains used in this study were isolated from humans in France and Belgium who acquired salmonellosis during or immediately after travel to Egypt, Libya, Sudan, and Kenya (11, 44). These isolates were previously described in studies reporting the emergence of ciprofloxacin-resistant serovar Kentucky isolates since 2002 in travelers returning from Northeastern and Eastern Africa (11, 44). In addition to ciprofloxacin resistance, some strains displayed different MDR phenotypes (11, 44). The serovar Kentucky strains and control strains *S. enterica* serovar Typhimurium DT104 BN9181 (SGI1) and serovar Newport 01-2174 (SGI1-H) and *E. coli* strain TOP10 used in cloning experiments were grown at 37°C in brain heart infusion or Luria-Bertani broth or agar plates. The strains were tested for their antibiotic susceptibilities by the disk diffusion assay on Mueller-Hinton plates according to the guidelines of the Antibiogram Committee of the French Society for Microbiology (30). Susceptibility was determined using disks containing 32 antibiotics as previously described (44). All antibiotic disks except for florfenicol were purchased from Bio-Rad (Marnes-la-Coquette, France). Florfenicol disks were obtained from Schering-Plough Animal Health (Kenilworth, NJ).

PCR mapping and Southern blot hybridization. Detection of SGI1 and its chromosomal location was performed using primers corresponding to the left and right junctions in the chromosome as described previously (Table 1 and Fig. 1) (4). PCR mapping of the 5′ region of SGI1 (the first 10 kb) was performed using PCRs 3 to 9 and the corresponding primers described in Table 1 (Fig. 1). The presence of the remaining non-MDR region of SGI1 was assessed by Southern blot hybridization of whole genomic DNA cut by XbaI (Promega, Charbonnières, France) by using probe p1-9 as previously described (4). This probe corresponds to a 2-kb EcoRI fragment comprising parts of the S023 and S024 open reading frames (ORFs).

To assess variant SGI1 MDR regions identified in this study, PCR mapping was performed using PCRs 10 to 24 and the corresponding primers described in Table 1 (Fig. 2). Some combinations of primers of different PCRs were also used to characterize local rearrangements in variant SGI1 MDR regions. The variants SGI1-K1, -K2, and -K3 were also assessed by Southern blotting of genomic DNA

TABLE 1. Primers used for PCR in this study

Primer	Target gene	Position in SGI1[a]	PCR	Nucleotide sequence (5′–3′)	Source or reference
Linker1			LM-PCR	TAATTACACGTTACGACTTCAGATC	This study
Linker2			LM-PCR	GATCTGAAGTCGTAACGTG	This study
CS1	Cassette array			GGCATCCAAGCAGCAAG	24
CS2	Cassette array			AAGCAGACTTGACCTGA	24
U7-L12	*thdF*	1	1	ACACCTTGAGCAGGGCAAAG	4
LJ-R1	*int*	500	1	AGTTCTAAAGGTTCGTAGTCG	4
104-RJ	S044	42373	2	TGACGAGCTGAAGCGAATTG	4
104D	*yidY*	47130	2, 24	ACCAGGGCAAAACTACACAG	4
int-Fw	*int*	393	3, 5	CCCTAACTCGAAGGGGCTCC	This study
int-Rv	*int*	1565	3	ACGGACTTTCCGCAGTGAGG	This study
xis-Fw	*xis*	1927	4	CCTTGAATGCCTTGAGAAGC	This study
xis-Rv	*xis*	1472	4	GTCGCCGGGTAACAGCAATG	This study
RvS004	S004	3561	5	CCAAATAGGGCACTTCCAGA	This study
FwtraN	*traN*	3969	6, 8	GAGAGAGTGGCGAGTTGAAA	This study
RvtraN	*traN*	6034	6	GTGATGCTGATGCTATGGAT	This study
FwS006	S006	6335	7	CATAGGAGCGAGGCAACAAA	This study
RvS008	S008	8035	7	CGCCTTTTTCATTTACGCCT	This study
FwtraG	*traG*	8673	9	CTTTCAATAATCGCAGCCAT	This study
RvtraG1	*traG*	11784	9	GCTTGTTGGCTCGTGTATGC	This study
RvtraG2	*traG*	8692	8	ATGGCTGCGATTATTGAAAG	This study
FwS026	S026	25116	10	CGGTTTTGAATAAGAAGGCA	This study
Fresol	*res*	26429	11	TCGGGTCTAGCGGCATTCTT	15
Rvaac3Id	*aac(3)-Id*		10	GCTCTATGGCTGGTTGGTCT	This study
FwaadA7	*aadA7*		12	TCGTGAAGTATGAAGCAGTT	This study
sulTER	*sul1*	29604	13	AAGGATTTCCTGACCCTG	15
Rsul1	*sul1*	39383	11	CGACACCGAGACCAATAGCG	15
FwIS6100	IS*6100*	40820		TGAGGAAATGCTGGCGGAAC	This study
RvIS6100	IS*6100*	41481	12	CTGCGAAATGGTGGTTGAGC	This study
FwTn501	orf2Δ		14	ACTGACGAAGACGGCGAATG	This study
RvTn501	orf2Δ		13	ATGGATGTGGTGGCTGAAGG	This study
RvRH389	*merA*		14	GTGCCGTCCAAGATCATG	27
FwRH446	*merA*		15	CTTACTGCGGTCAATCGTAGG	27
RvRH447	*merR*		15	CACACCAACTCAGACAGCACG	27
FwmerRb	*merR*		16	TGCTGTCTGAACTCGTGTGCG	This study
RvtetA	*tetA*		16	CGGCAGGCAGAGCAAGTAGA	This study
FwtetR	*tetR*		17	CCAGTTTGCGTGTCGTCAGA	This study
RvpecM	*pecM*		17	GCGGGTTTATTGCTCGTGAT	This study
FwpecM	*pecM*		18	AGGACGCCGATGATTTGAAG	This study
FwtnpA	*tnpA*		19	AACCCGAACAGCGACCAGTA	This study
RvtnpA	*tnpA*		18	CGGTTAGGTTGATGTGCTCC	This study
FwstrB	*strB*		20	CCCGTCTGGCAATGAAACTT	This study
RvstrA	*strA*		19	ATTGCTAACGCCGAAGAGAA	This study
FwIS1133	IS*1133*		21	CGCCAAATCAACTCAGCCTT	This study
RvIS1133	IS*1133*		20	CTTCGGGAGGAGAGGGAGAT	This study
RvtnpRa	*tnpR*			TCAGCAAATCCAGCCCTTCCC	This study
FwIS26	IS*26*		21, 24	GCACGCATCACCTCAATACC	This study
RvIS26	IS*26*		22	TCACTCCACGATTTACCGCT	This study
OT3	bla_{TEM-1}		23	ATGAGTATTCAACATTTCCG	17
OT4	bla_{TEM-1}		22	CCAATGCTTAATCAGTGAGG	17
FwS044	S044	42200		CTACCCAGGAGCCACAATCA	This study
RvS044	S044	41924		TAACTCTCTCTGGTGTGTCG	This study
RvS044b	S044	42231		TTGTCGTCGGGATGATTGTGG	This study
RvS044c	S044	42234	23	GTATTGTCGTCGGGATGATT	This study

[a] Primer positions are given according to the complete SGI1 sequence (GenBank accession no. AF261825) (3).

cut by HpaI and MluI (Promega) by using the insert of recombinant plasmid pBD14, comprising a large fragment of the variant SGI1-K1 MDR region, as a probe.

LM-PCR, cloning, and sequencing. To assess the 3′ part of variant SGI1-K MDR regions identified in this study, ligation-mediated PCR (LM-PCR) was done as described below. Genomic DNAs of serovar Kentucky strains were cut by blunt-end restriction enzyme SmaI (Promega). Annealing of the two primers Linker1 and Linker2 (Table 1) to form the double-stranded adaptors was performed by boiling a 5 nM solution of the mixed primers, followed by slow cooling to room temperature. SmaI-digested chromosomal DNAs were ligated to the blunt-end adaptors (Linker1 and Linker2) in a 10-μl final volume at a 10-fold molar excess of the adaptor according to the number of generated fragments. A first round of amplification was performed by using primer Linker1 and primer RvS044c to the right end of the SGI1-K variant (Table 1) in 25-μl PCR mixtures with a GoTaq Master Mix kit (Promega) and 2 μl of ligation mixture. The first-round PCR conditions were (i) 5 min at 95°C; (ii) 30 cycles of 30 s at 95°C, 30 s at 60°C, and 5 min at 72°C; and (iii) a final extension step of 7 min at 72°C. The second round of amplification was performed like the first round, with 2 μl of the first-round reaction mixture as the template and primers Linker1 and RvS044b, which was identical to the leftmost end of ORF S044 (Table 1). The purified PCR products were sequenced by using primer RvS044b at Genome Express (Meylan, France) and were compared with the GenBank DNA sequence

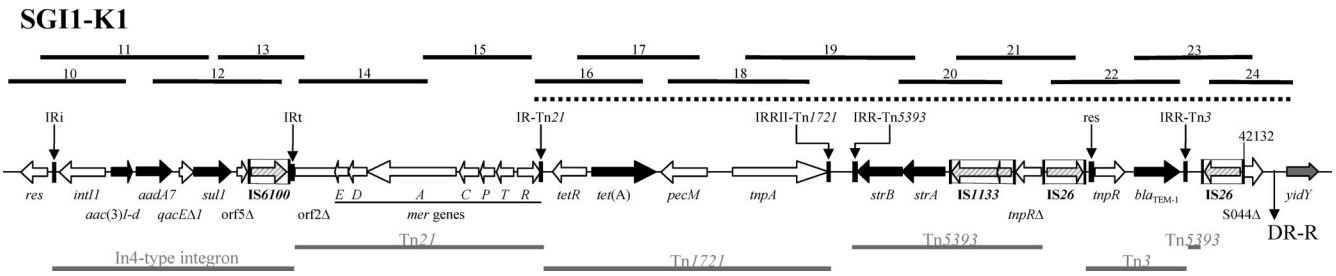

FIG. 2. Schematic view of the SGI1-K1 MDR region. Antibiotic resistance genes and IS elements are indicated by black arrows and gray hatched arrows within boxes, respectively. The different inverted repeats and resolution sites of transposon or integron sequences are indicated by vertical black bars. DR-R represents the right 18-bp direct repeat at the end of SGI1. Base pair coordinates are from the complete SGI1 sequence (GenBank accession no. AF261825). PCRs carried out to map the SGI1-K1 MDR region are indicated by thick black bars and are numbered (Table 1). The black dotted line indicates the sequenced region. Gray labeled lines at the bottom represent regions in which the sequences exhibit significant homology to extant sequences on various genetic elements. The GenBank accession numbers of the sequence used for analysis are given in the text.

database by using the genomic BLASTN program (available at http://www.ncbi.nlm.nih.gov/blast).

To characterize the remaining parts of the MDR regions of the SGI1-K1 (strain 01-2100) and SGI1-K2 (strain 05-5572) variants, cloning of PCR products using primer pairs FwmerRb-RvtnpRa and Fresol-Rsul1, respectively, in plasmid PCR2.1-TOPO was performed using the TOPO TA cloning kit (Invitrogen, Cergy Pontoise, France). The recombinant plasmids obtained were named pBD13 and pBD14, respectively. Nucleotide sequencing of the pBD13 and pBD14 inserts and other PCR products was achieved by use of Genome Express. Sequence analysis was done by using BLASTN.

Nucleotide sequence accession numbers. The complete nucleotide sequence from the SGI1-K1 variant reported in this paper has been deposited in the GenBank database under accession number EU664602 according to the previously published SGI1-K variant (GenBank accession no. AY463797) (27) and the complete nucleotide sequence of SGI1 (GenBank accession no. AF261825) (4). The nucleotide sequences of different regions from SGI1-K2, SGI1-K3, SGI1-K4, SGI1-K5, SGI1-P1, SGI1-P2, SGI1-Q1, SGI1-Q2, and SGI1-Q3 have also been deposited in the GenBank database under accession numbers EU664603, EU664604, EU664605, EU664606, EU664607, EU664608, EU664609, EU664610, and EU664611, respectively.

RESULTS

Detection of SGI1 in serovar Kentucky strains isolated from travelers returning from Africa and MDR profiles. Sixteen serovar Kentucky strains resistant to at least one antibiotic and isolated from humans in France and Belgium who acquired salmonellosis during or immediately after travel to Egypt, Libya, Sudan, and Kenya between 2001 and 2005 were analyzed for the presence of SGI1 (Table 2). The detection and location of SGI1 in the *Salmonella* chromosome were performed by junction PCRs 1 and 2 (Table 1 and Fig. 1). SGI1 was thus detected in 14 serovar Kentucky strains. It was integrated into its specific attachment site between the *thdF* and *yidY* genes of the chromosome. Two serovar Kentucky strains resistant to nalidixic acid and tetracycline, respectively, were negative for the presence of SGI1 and showed an intact region between the *thdF* and *yidY* genes. All serovar Kentucky strains lacked the retron sequence, which is located downstream of SGI1 in serovar Typhimurium DT104 strains but absent in other serovars reported to date (3, 4, 16, 34). Interestingly, among the 14 serovar Kentucky strains positive for SGI1 junctions (PCRs 1 and 2), strain 05-1016 was resistant to quinolones and fluoroquinolones only, and strains 00-1059, 05-0520, and 05-2131 harbored resistance only to ampicillin in addition to quinolone and/or fluoroquinolone resistance (Table 2). The other SGI1-positive strains displayed various MDR profiles in addition to quinolone and fluoroquinolone resistance, from 3 to 10 antibiotics (Table 2). The possible occurrence of SGI1 variant MDR regions in these serovar Kentucky strains was assessed by PCR mapping, cloning, and sequencing.

Genetic characterization of the non-MDR region of SGI1 in serovar Kentucky strains. The first 10 kb of SGI1 was assessed by PCR mapping (PCRs 3 to 9) as described in Table 1 and Fig. 1. All strains yielded fragments for PCRs 3, 4, 5, and 9 of the sizes expected from serovar Typhimurium DT104 control strain BN9181 harboring SGI1. However, fragments 6 and 7 were not obtained for all serovar Kentucky strains tested (Fig. 1). These results suggested a genetic rearrangement in the region spanning from ORF S005 (*traN* homolog) to ORF S011 (*traG* homolog). To assess this region, PCR 8 was undertaken using the forward primer of PCR 6 (FwtraN) and a reverse primer (RvtraG2) located at the end of S011. All serovar Kentucky strains tested yielded the same fragment, around 3.2 kb in size, which was 1.5 kb smaller than that of serovar Typhimurium DT104 control strain BN9181 (data not shown). Strain 01-2100 was chosen as a representative strain, and its 3.2-kb PCR product was sequenced (GenBank accession no. EU664602). Sequence analysis identified a deletion of 2,780 bp in size from part of ORFs S005 to S009 (Fig. 1). This deletion in SGI1 was replaced by an insertion sequence (IS) element of 1,257 bp (Fig. 1). Sequence analysis using BLASTN identified that this IS element showed 99% nucleotide identity to IS*1359* (GenBank accession no. AF179596), also called IS*Vch4* in the IS database (at http://www-is.biotoul.fr) described for *Vibrio cholerae* (8). This IS belongs to the IS*3* family (6, 28). Thus, this IS element inserted into SGI1 of serovar Kentucky strains was named IS*Vch4* (Fig. 1). Interestingly, we did not find direct repeats representing the target site duplications usually created upon the insertion of the IS element (GenBank accession no. EU664602).

Downstream of this genetic rearrangement, the presence of a large central region of SGI1 from bases 11949 to 24937 (GenBank accession no. AF261825) in serovar Kentucky strains was confirmed by Southern blot hybridization of XbaI-digested genomic DNA with probe p1-9 as described previously (4). Two XbaI fragments of 4 kb and 9 kb hybridized with the probe in all SGI1 serovar Kentucky strains (data not

TABLE 2. SGI1-carrying *S. enterica* serovar Kentucky strains characterized in this study

Strain[a]	Country	Antibiotic resistance profile[b]	Integron-borne gene cassettes[c]	PCR mapping result for SGI1 MDR region[d,e]:															SGI1 variant[f]
				10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
01-2100	Egypt	**Amp Gen Str Spt Sul Tet-**Nal	*aac(3)-Id–aadA7*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	SGI1-K1
02-8141	Egypt	**Amp** Chl Flo **Gen Str Spt Sul Tet-**Nal	*aac(3)-Id–aadA7*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	SGI1-K1
04-4567	Egypt	**Amp** Chl **Gen** Kan Neo **Str Spt Sul Tet** Tmp-Nal Flu Cip Enr	*aac(3)-Id–aadA7 dfrA12-*orfF*-aadA2*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	SGI1-K1
04-7734	Unknown	**Amp** Chl **Gen** Kan Neo **Str Spt Sul Tet** Tmp-Nal Flu	*aac(3)-Id–aadA7 dfrA12-*orfF*-aadA2*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	SGI1-K1
05-5111	Libya	**Amp Sul Tet-**Nal Flu Cip Enr		−	+*	−	+	+	+	+	+	+	+	−	−	+	+	+	SGI1-K2
05-5572	Libya	**Amp Sul Tet** Tmp-Fep Caz Cro Ctx-Nal Flu Cip Enr		−	+*	−	+	+	+	+	+	+	+	−	−	+	+	+	SGI1-K2
02-2691	Egypt	**Amp Gen Str Spt Sul Tet-**Nal	*aac(3)-Id–aadA7*	−	−	+	+	+	+	+	+	+	+	+	+	+	−	+	SGI1-K3
05-4680	Sudan	**Gen Str Spt Sul Tet** Tmp-Nal Flu Cip Enr	*aac(3)-Id–aadA7 dfrA12-*orfF*-aadA2*	+*	+*	+	+	+	+	+	−	−	−	−	−	−	−	+	SGI1-K4
04-8262	Egypt	**Gen Str Spt Sul-**Nal Flu Cip Enr	*aac(3)-Id–aadA7*	+	+	+	+	+	+	−	−	−	−	−	−	−	−	+	SGI1-K5
00-1059	Egypt	**Amp-**Nal		−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	SGI1-P1
05-0520	Egypt	**Amp-**Nal Flu Cip Enr		−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	SGI1-P2
05-2131	Egypt	Amp-Nal Flu Cip Enr		−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	SGI1-Q1
05-1016	Kenya	Nal Flu Cip Enr		−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	SGI1-Q2
05-1199	Egypt	Gen Str Spt Sul Tet-Nal Flu Cip Enr	*aac(3)-Id–aadA7*	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	SGI1-Q3

[a] The year of isolation of the strain is indicated by the first two numbers.
[b] Resistance to antibiotics indicated in boldface type is conferred by SGI1. Abbreviations: Amp, ampicillin; Chl, chloramphenicol; Flo, florfenicol; Gen, gentamicin; Kan, kanamycin; Neo, neomycin, Str, streptomycin; Spt, spectinomycin; Sul, sulfonamides; Tet, tetracycline; Tmp, trimethoprim; Nal, nalidixic acid; Flu, flumequine; Cip, ciprofloxacin; Enr, enrofloxacin; Fep, cefepime; Caz, ceftazidime; Cro, ceftriaxone; Ctx, cefotaxime.
[c] *aac(3)-Id–aadA7* and *dfrA12-*orfF*-aadA2* cassette arrays are 1,579 bp and 1,913 bp in size, respectively.
[d] +* indicates a positive result but with different sizes of amplicons.
[e] See Fig. 2 for PCR numbering.
[f] See Fig. 2, 3, and 4.

shown). It was concordant to the SGI1 nucleotide sequence (GenBank accession no. AF261825) and identical to what is observed in other SGI1-carrying *S. enterica* serovars.

Complete characterization of the SGI1-K MDR region. At first, gene cassette arrays of serovar Kentucky strains were determined by PCR using primers CS1 and CS2 in the integron 5′-CS and 3′-CS (Table 1) (24). Three serovar Kentucky strains harbored two cassette arrays of 1.6 kb and 2 kb, five strains harbored a single 1.6-kb cassette array, and no amplification products were observed in six strains (Table 2). The 1.6-kb and 2-kb cassette arrays were sequenced. Sequence analysis identified the resistance gene cassettes *aac(3)-Id* (coding for gentamicin resistance) and *aadA7* (coding for streptomycin and spectinomycin resistance) in the 1.6-kb array and the resistance gene cassettes *dfrA12* (coding for trimethoprim resistance), orfF (unknown function), and *aadA2* (coding for streptomycin and spectinomycin resistance) in the 2-kb array. This 2-kb cassette array was not found to be associated with SGI1 (see the complete characterization of SGI1 variants) and, thus, was probably located on plasmids or elsewhere in the chromosome (data not shown). The 1.6-kb cassette array in SGI1-H (GenBank accession no. AY458224) found in serovar Newport was previously described (14), and that in SGI1-K (GenBank accession no. AY463797) for serovar Kentucky was recently described (27). According to the partial sequence of the MDR region of SGI1-K described previously by Levings et al. (27), we performed PCR mapping from the SGI1 backbone at the left boundary of the integron to the mercury resistance module (*mer* module) found downstream of the IS*6100* element (PCRs 10 to 15) (Table 1 and Fig. 2) (27). Five serovar

Kentucky strains yielded fragments 10 to 15, of the sizes expected from the SGI1-K sequence (GenBank accession no. AY463797) (Table 2 and Fig. 2). It was suggested that these serovar Kentucky strains harbored the *aac(3)-Id–aadA7* integron-borne cassettes associated with a *mer* module (27). Four other serovar Kentucky strains tested were partially positive for this PCR mapping and/or yielded fragments 10 and/or 11 of unexpected sizes. The remaining strains were negative for PCRs 10 to 15 (Table 2). The SGI1 characterization of the strains harboring genetic variations in this region is described in the two next sections.

To assess the 3′ right end of SGI1-K, we examined the upstream S044 region of representative strain 01-2100 by LM-PCR as described in Materials and Methods. A 2.7-kb fragment was obtained and was sequenced. The insertion of an IS*26* element within the 5′ left end of ORF S044 was identified (Fig. 2). Upstream of the IS*26* element, a region corresponding to the 3′ right end of a Tn*3*-like element containing the β-lactamase gene $bla_{\text{TEM-1}}$ and the 3′ end of the Tn*3 tnpR* gene was found (Fig. 2). This result suggested that the variant SGI1-K MDR region had been subjected to several events resulting in such rearrangements.

Next, to fully characterize the SGI1-K variant of representative strain 01-2100, we attempted to establish the linkage between the *mer* module and the 3′ end of the *tnpR* gene by long-range PCR. Using primers FwmerRb and RvtnpRa, a single fragment around 10 kb in size was obtained. This PCR product was cloned and sequenced. The recombinant plasmid obtained was named pBD14 and interestingly conferred resistance to streptomycin, spectinomycin, and tetracycline in a susceptible *E. coli* host strain. As expected from the SGI1-K variant, the 10,610-bp insert contained the 3′ end of the *merR* gene and the right inverted repeat of Tn*21* (IR-Tn*21* in Fig. 2) (GenBank accession no. AF071413). Downstream of IR-Tn*21*, a 5-kb region of Tn*1721* contained the tetracycline resistance genes *tetR* and *tet*(A), the *pecM* gene, a *tnpA* gene, and the second right inverted repeat of Tn*1721* (Fig. 2) (GenBank accession no. X61367). Another region of a transposon, named Tn*5393*, was found downstream of Tn*1721* and included the streptomycin resistance genes *strAB*, an IS*1133* element, and the 3′ end of the *tnpR* gene of Tn*5393* (Fig. 2) (GenBank accession no. M95402). The 5′ end of the Tn*5393 tnpR* gene was replaced by the insertion of an IS*26* element (Fig. 2). Downstream of this IS*26* element, the 5′ part of the Tn*3*-like *tnpR* gene was found, ending this 10,610-bp insert of pBD14. Thus, this sequence was gathered with the sequence of the 3′ end of SGI1 obtained by LM-PCR. The resulting sequence of serovar Kentucky strain 01-2100, which completes the variant SGI1-K MDR region previously described (GenBank accession no. AY463797) (27), was used to construct the map of variant SGI1-K1 in Fig. 2 and deposited in GenBank under accession number EU664602. The nomenclature of SGI1 variants was slightly modified by adding a number to the letter according to the genetic rearrangements in the SGI1-K MDR region described below. The mapping of this novel MDR region of SGI1-K1 was done for the other serovar Kentucky strains, and thus, three strains yielded fragments 16 to 24 of the sizes expected from the SGI1-K1-carrying strain 01-2100 (Table 2). The global genetic organization of the SGI1-K1 variant MDR region in the SGI1 backbone was confirmed by Southern blotting of HpaI- and MluI-double-digested genomic DNAs with the pBD14 insert used as a probe (data not shown). Currently, this new variant SGI1-K1 MDR region of around 23 kb in size and containing seven different antibiotic resistance genes represents the largest MDR gene cluster described in SGI1. Moreover, it is the first occurrence of chromosomally encoded β-lactamase gene $bla_{\text{TEM-1}}$ in *Salmonella*.

Genetic rearrangements in variant SGI1-K MDR regions. The complete PCR mapping (PCRs 10 to 24) of the variant SGI1-K1 MDR region revealed some genetic rearrangements for several SGI1-K-carrying serovar Kentucky strains. Strains 05-5111 and 05-5572 showed the same two modifications compared to the structure of SGI1-K1. The first one was revealed by the absence of integron-borne cassettes, the absence of amplification for PCR 10, and small PCR products for PCR 11 (2.5 kb) compared to that of strain 01-2100 (4 kb). The PCR product was cloned and sequenced (GenBank accession no. EU664603). The recombinant plasmid obtained was named pBD13. Sequence analysis identified the insertion of an IS*26* element downstream of the IRi of the integron. The left inverted repeat of IS*26* abuts within the *aadA7* gene cassette (Fig. 3). The integrase gene *intI1* and the *aac(3)-Id* and the 5′ end of the *aadA7* gene cassettes were shown to be deleted. The second genetic rearrangement in strains 05-5111 and 05-5572 occurred in the region of IS*1133* (Fig. 3). PCRs 21 and 22 were negative in repeated attempts; thus, PCR was done using the forward primer of PCR 20 and the reverse primer of PCR 21. The resulting 1.8-kb PCR product was sequenced (GenBank accession no. EU664603). Sequence analysis revealed that the *strA* gene was deleted in its 5′ end due to the insertion of a partial IS*26* element also deleted by another complete IS*26* (Fig. 3). This variant of the SGI1-K1 MDR region was named SGI1-K2.

Strain 02-2691 was positive for a great majority of the PCRs (PCRs 12 to 22 and 24). However, the left and right boundaries of the SGI1-K MDR region seemed to be subjected to genetic rearrangements according the absence of amplification products for PCRs 10, 11, and 23. In this strain, these boundaries were sequenced (GenBank accession no. EU664604). The SGI1-K MDR region was inverted between the IS*26* element inserted into S044 and another IS*26* element inserted into the *res* gene of SGI1. This inversion of the SGI1-K1 MDR region was named SGI1-K3 (Fig. 3).

Strains 05-4680 and 04-8262 were positive for the 5′ part of the MDR region containing the integron and the *mer* module (Table 2 and Fig. 3). However, the 3′ end was absent in these two strains. Two different deletions extended from the IS*26* element inserted into S044 to the *pecM* gene or to the tetracycline resistance gene *tetA*. Moreover, in strain 05-4680, another IS*26* element was found inserted into the 3′ end of the *intI1* gene. The SGI1-K variants of strains 05-4680 and 04-8262 were named SGI1-K4 and -K5, respectively (Fig. 3) (GenBank accession no. EU664605 and EU664606).

Newly discovered SGI1 variants with restricted MDR regions or without MDR regions. The five remaining serovar Kentucky strains were negative for almost all the PCRs (Table 2 and Fig. 2). However, all these strains yielded fragment 24 of the expected size of 1.4 kb, indicating the insertion of IS*26* into S044. Strains 00-1059 and 05-0520 were also positive for PCRs 22 and 23 (Fig. 2), indicating that the $bla_{\text{TEM-1}}$ gene flanked by

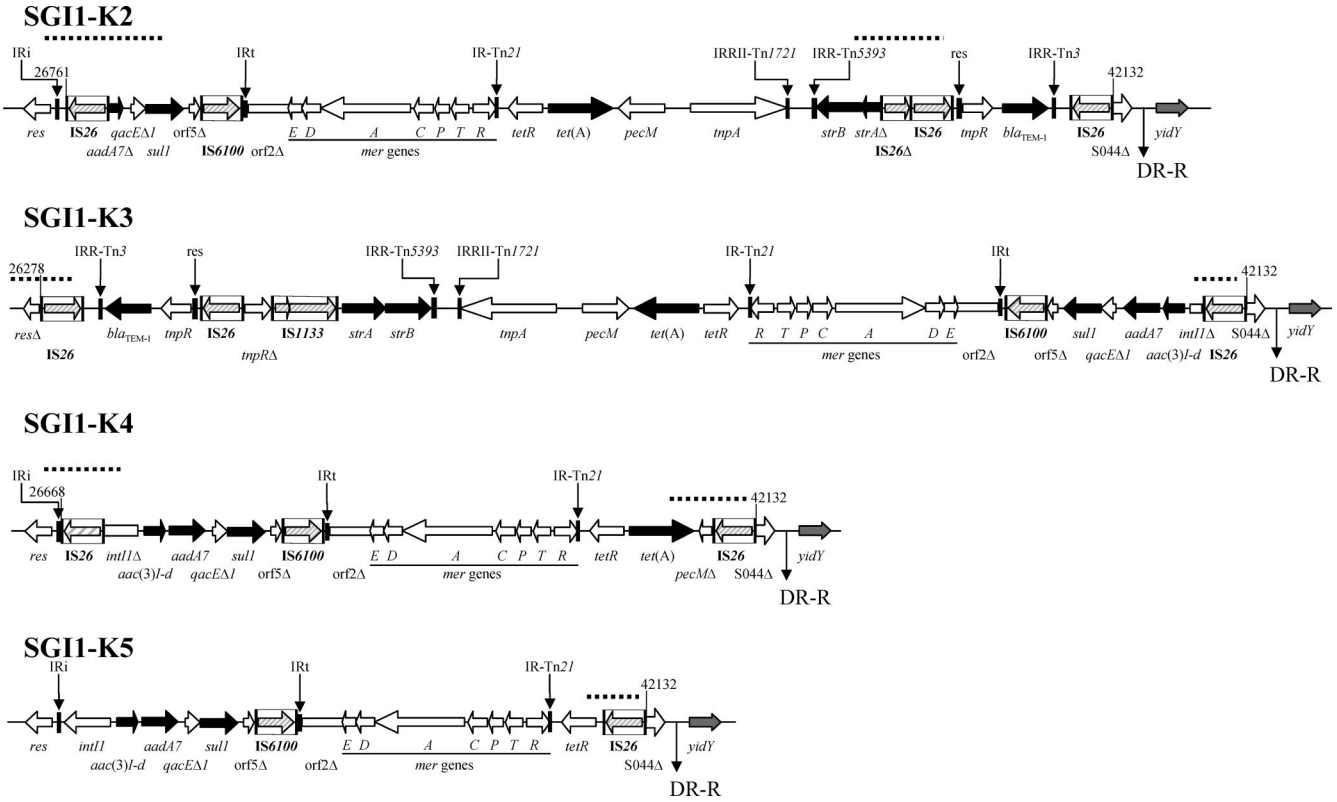

FIG. 3. Schematic views of the SGI1-K2, -K3, -K4, and -K5 MDR regions. Antibiotic resistance genes and IS elements are indicated by black arrows and gray hatched arrows within boxes, respectively. The different inverted repeats and resolution sites of transposon or integron sequences are indicated by vertical black bars. DR-R represents the right 18-bp direct repeat at the end of SGI1. Base pair coordinates are from the complete SGI1 sequence (GenBank accession no. AF261825). The sequenced regions are indicated by black dotted lines.

two IS*26* elements in opposite orientations was located at the 3′ end of the SGI1 MDR region. The linkage with the SGI1 backbone was demonstrated by PCR using forward primer Fresol and FwIS26 or OT4 as a reverse primer (Table 1). The two Fresol-FwIS26 PCR products differed in size (1,000 bp versus 900 bp) were sequenced to localize the precise insertion of the left IS*26* element in SGI1 (Fig. 4) (GenBank accession no. EU664607 and EU664608). These two different variant SGI1 MDR regions conferring only ampicillin resistance were named SGI1-P1 and -P2, respectively (Table 2 and Fig. 4).

The three strains 05-2131, 05-1016, and 05-1199, which were positive only for PCR 24, were suspected to harbor a more restricted region containing only one IS*26* element. Thus, these strains were tested by PCR using forward primer Fresol and reverse primer 104D (Table 1). These strains yielded PCR products of different sizes (1.5 kb, 1.7 kb, and 2.0 kb), which were

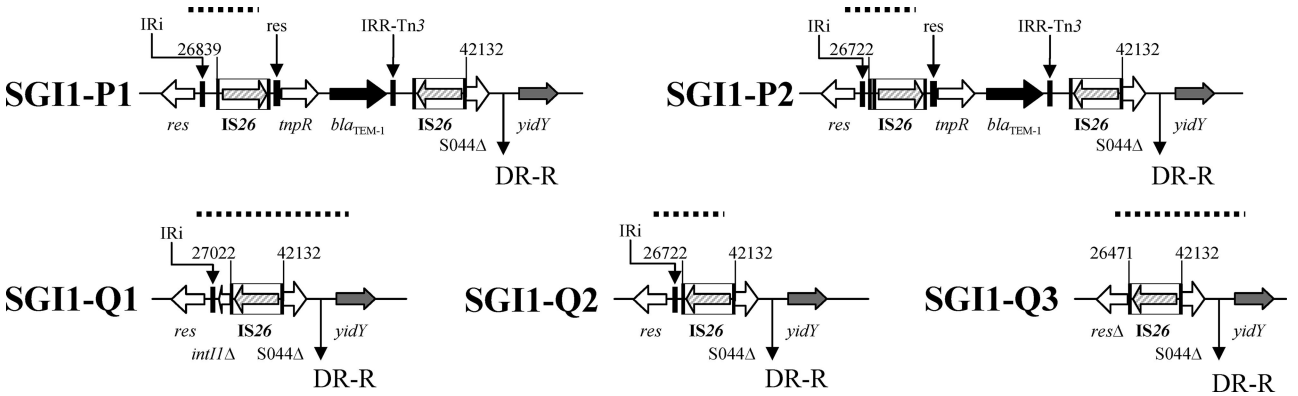

FIG. 4. Schematic views of the SGI1-P1, -P2, -Q1, Q2, and -Q3 MDR regions. Antibiotic resistance genes and IS elements are indicated by black arrows and gray hatched arrows within boxes, respectively. The different inverted repeats and resolution sites of transposon or integron sequences are indicated by vertical black bars. DR-R represents the right 18-bp direct repeat at the end of SGI1. Base pair coordinates are from the complete SGI1 sequence (GenBank accession no. AF261825). The sequenced regions are indicated by black dotted lines.

sequenced (GenBank accession no. EU664609, EU664610, and EU664611). Sequence analysis identified different left insertion sites of the IS*26* element in the region encompassing the *res* gene to the 5′-CS of the integron (Fig. 4). These new variant SGI1 MDR regions, which did not contain any antibiotic resistance genes, were named SGI1-Q1 to -Q3 (Fig. 4 and Table 2).

DISCUSSION

SGI1 is the first genomic island containing an antibiotic resistance gene cluster identified in *S. enterica*. Its acquisition may have been an important trait to the worldwide epidemic of *S. enterica* serovar Typhimurium DT104 clone causing disease in animal as well as in humans (10, 34). SGI1 has been further identified in 14 *S. enterica* serovars and also recently in *Proteus mirabilis* (1, 5, 23, 34). In 2005, the horizontal transfer and integration specificity of SGI1 has been experimentally demonstrated between different *S. enterica* serovars and between *S. enterica* and *E. coli* (12). Recently, Levings et al. partially characterized the variant SGI1-K MDR gene cluster, which conferred gentamicin, streptomycin, spectinomycin, and mercury ion resistance (26, 27).

Since the end of 2002, the emergence of multidrug-resistant and ciprofloxacin-resistant serovar Kentucky strains isolated from European travelers returning from African countries was reported in different studies (11, 29, 32, 44). The SGI1-carrying serovar Kentucky strains studied here presented different MDR phenotypes with or without resistance to ciprofloxacin (Table 2).

Interestingly, all SGI1 variants in serovar Kentucky strains studied presented the same genetic rearrangement in the 5′ end of the SGI1 backbone (Fig. 1). The deletion of an SGI1 segment and its replacement by an IS*Vch4* element, also called IS*1359*, from *Vibrio cholerae* suggested that genetic exchange may have occurred between SGI1 and *Vibrio* spp. Such events had also been suspected by the identification of a short DNA segment of SGI1 integrated into the ICE_{SXT} of *V. cholerae* and also by the concomitant description of the *aac(3)-Id–aadA7* cassette array in *S. enterica* and *Vibrio fluvialis* strains (2, 14, 22, 25, 26). The presence of IS*Vch4* inserted into the SGI1 backbone indicates that all different variants have probably evolved from a common SGI1 ancestor that had acquired this IS*Vch4*.

The different SGI1 variants characterized in the present study showed several genetic changes compared to the previously described variants SGI1-A to -O (5, 9, 27, 43). In all these variant SGI1-K, SGI1-P, and SGI1-Q MDR regions, the IS*26* elements may have played an important role in their generation. IS*26* elements are often found to be associated with antibiotic resistance genes and transposon structures (18, 31, 35, 38, 45). The SGI1-K1 MDR region shares 99% nucleotide identity with different segments of MDR plasmids carrying transposons such as the Tn*21*, Tn*1721*, Tn*5393*, and Tn*3*-like transposons (Fig. 2) (GenBank accession no. AF071413, X61327, M95402, and X54607, respectively). These mobile elements harboring antibiotic resistance genes are found in a complete or truncated form on numerous large MDR plasmids of different sources. These large MDR plasmids have been described for different bacterial species such as *Salmonella enterica* (serovars Typhi, Paratyphi A, Typhimurium, Newport, and Dublin), *Yersinia pestis*, *Klebsiella pneumoniae*, and IncP-1β plasmids from a wastewater treatment plant (7, 20, 38, 40, 41, 45). Interestingly, parts of Tn*21*, Tn*1721*, Tn*5393*, In4-type integron structures, and IS*26* elements are also found in an 86-kb chromosomal resistance island recently identified in multidrug-resistant *Acinetobacter baumannii* strain AYE (18). This resistance island, named AbaR1, shares several similarities in the organization of the different mobile elements with SGI1-K MDR regions (18). Moreover, all these large MDR regions also harbor several IS*26* elements linked to antibiotic resistance genes and composite transposon structures (7, 18, 20, 38, 40, 41, 45).

Interestingly, in all SGI1-carrying serovar Kentucky strains studied, the rightmost IS*26* in SGI1-K and SGI1-P variants and also the unique IS*26* of SGI1-Q variants were always found inserted at the same precise position within S044 of the SGI1 backbone. These results indicated that in the evolution of these SGI1 variants, there was probably an event resulting in the insertion of an IS*26* element in S044. Different steps of intermolecular homologous recombination between this potential SGI1 ancestor and different large MDR plasmids could then explain the generation of the SGI1-K1 MDR region in serovar Kentucky. Interestingly, in the SGI1-K1 sequence, as well as in SGI1-K2 and -K3 between the Tn*1721* and Tn*5393* sequence, a short segment corresponds to a part of the *fipA* gene, encoding a fertility inhibition protein of IncP plasmids. Interestingly, in all SGI1-K, -P, and -Q MDR regions, none of the IS*26* elements showed target site duplications (21, 33). Moreover, this absence of a direct duplication of a target sequence at each side of these SGI1 MDR regions indicates that they were probably not inserted by a transpositional mechanism as described previously for class II transposons or IS*26* composite transposons (6, 19, 21, 28, 31, 35). All these data strengthen the hypothesis of homologous recombinations between these SGI1 MDR regions and different MDR gene clusters of distinct replicons.

The IS*26* element inserted into the SGI1 backbone (S044) probably facilitated the recruitment of diverse IS*26*-flanked sequences such as Tn*3*, Tn*5393*, or In4-type integrons. The properties of the IS*26* cointegration of different replicons could explain the mobilization of different IS*26*-flanked sequences (6, 19, 21, 28, 31). IS*26* is widely spread among plasmids and could be implicated in the acquisition of resistance genes in different ways. IS*26* composite transposons carrying from one to nine antibiotic resistance genes were described previously (21, 31, 35). Their transposition generates 8-bp target site duplications. Transpositions and/or homologous recombinations of IS*26* elements associated with integrons and transposon structures are probably implicated in the building of the SGI1 MDR regions studied here and could represent the initial steps in the generation of different more or less large composite IS*26* transposons in SGI1 as well as in plasmids (31).

A recent study described a new transposon, Tn*2610*, found in a conjugative plasmid from an *E. coli* strain isolated in 1975 in Japan (42). Tn*2610* is bracketed by two transposition modules, Tn*1721* and Tn*21*. Interestingly, part of the SGI1 In104 integron is found in this transposon, originally isolated before SGI1-containing strains (4, 42). This transposon probably originated from Tn*1721* and Tn*21*, with extensive recombination events with other elements, which have resulted in a complex mosaic structure. Antibiotic resistance gene exchange, carried by plasmids, resistance islands, transposons, and integrons, be-

tween different bacterial pathogens such as *Salmonella*, *Shigella*, *Vibrio*, *Klebsiella*, and *E. coli* of different sources may take place in the natural aquatic environment, where fecal contaminations often occurred in African countries (39).

In conclusion, for the first time, the variant MDR regions of SGI1 in this study seem to have evolved from MDR regions of different large plasmids or other resistance islands. It is also interesting that for the first time, the β-lactamase $bla_{\text{TEM-1}}$ gene was located in the *Salmonella* chromosome in SGI1-K1, -K2, K3, -P1, and -P2. Integron conserved segments and especially IS*26* elements are probably implicated in the generation of those SGI1 MDR regions. However, it remains difficult to estimate how the evolution of SGI1 MDR regions occurred between each other and whether one variant is at the origin of the other. The same insertions of the IS*Vch4* and IS*26* elements in the 5′ and 3′ parts of SGI1, respectively, indicate that all SGI1 islands of serovar Kentucky strains probably evolved from the same SGI1 ancestor. The insertion of an IS*26* element in the SGI1 backbone (S044) is probably the key feature in the building of variant SGI1-K, -P, and -Q MDR regions. The great plasticity of the MDR region of SGI1 associated with its ability of horizontal transfer could contribute to its spread and persistence among clinical enterobacteria.

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